Protein crystallography of muscle tropomyosin and complexes of tropomyosin with other proteins

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Tropomyosin, in association with troponin, plays the crucial role in the calcium-based regulatory mechanism of skeletal muscle contraction (Ebashi and Endo, 1968). It is also well known that tropomyosin isoforms are widely distributed in non-muscle cells, being always associated with actin and presumably making actin filaments more stable (Pittenger *et al.*, 1994). Tropomyosin has been a prominent member of α-helical coiled-coil proteins. The molecule is a rod 400 Å long and 20 Å thick, almost the entire length of which consists of an α-helical coiled-coil of two identical chains, 284 amino acids and about 33 kD.

The atomic structure of tropomyosin is of great interest because, on one hand, this molecule is a stable α-helical coiled-coil, and yet this molecule must be flexible enough to govern the states of many actin subunits in response to Ca^{2+}-binding to troponin. It is interesting to account for the flexibility of tropomyosin and the rigidity of the leucine zipper (O' Shea *et al.*, 1991), another α-helical coiled-coil protein.

Originally, we planed to measure the crystal formed from lobster slow muscle tropomyosin expressed in Sf9 insect cells, in the presence of 34 - 38 % DMSO. However, after a couple of sessions of beam time at ESRF in Grenoble, we found that this crystal form was not suitable for analysis due to the extreme anisotropic natuer of the diffraction pattern; the crystal diffracted X-ray beyond 3.0 Å resolution along *c**-axis, whereas along the *a**-*b**-plane only up to 6.6 Å resolution.

Just before the beam time at SPring-8 began, we obtained a new crystal form which was grown in the presence of 4.5 % PEGNME2kD (pH7.0) and 100 mM spermine. Because the crystals were too small (0.25 x 0.25 x 0.1 mm) to check on our lab source, we decided to check the crystals on the beam line spending only 2 hours, giving up the rest of the beam time to another group.

Although the crystals diffracted slightly better than those grown in DMSO, the new crystals were not yet good enough for starting data collection.